Regulation of Human Fetal and Adult Globin Genes in Mouse Erythroleukemia Cells

By B.J. Morley, C.A. Abbott, and W.G. Wood

We have examined whether transfected mouse erythroleukemia (MEL) cells can be used to examine differential expression of human γ and β-globin genes. These cells, which express only their adult globin genes, will transcribe the human adult β gene but not the fetal γ genes when they are introduced on an intact human chromosome 11 by cell fusion. However, MEL cells stably transfected with the human γγ gene inserted into an intact human chromosome 11 by cell fusion will transcribe the γ-globin locus control region (LCR) readily produce y-globin mRNA in amounts equivalent to those seen with a comparable β gene insert. When both β and γ genes are attached to HS2, equal amounts of β and γ mRNAs are produced, irrespective of the gene order. Furthermore, when the LCR, which binds to the cluster as known. High-level, position-independent expression of these genes is dependent on a region located 5′ to the cluster known as the locus control region (LCR), previously known as the dominant control region, or locus activating region (L) containing four DNase I hypersensitive sites (HS) spread over a distance of about 20 kb. There is as yet no evidence that these sequences play a direct role in developmental regulation, as they apparently activate all the genes within the cluster. While binding sites for several ubiquitous and erythroid cell-predominant trans-acting factors have been identified in promoter and enhancer regions around the genes and in the LCR, there is as yet no evidence for developmental stage-specific trans-acting factors in humans, although an adult erythroid cell-specific protein, which binds to the β-globin gene, has been reported in chickens.

In conditions in which large deletions remove the δ and β genes but spare the γ genes, there is persistence of γ gene expression into adult life (hereditary persistence of fetal hemoglobin HPFH) at levels up to 70% of the normal β gene output. Therefore, it follows that the trans-acting factors in adult erythroid cells are sufficient for high-level γ-globin gene transcription. This fact raises the possibility that normally the γ and β genes may be in competition for expression, such that in normal adult erythroblasts conditions favor expression of the β gene but in the absence of competition from the δ and β genes, the γ genes will continue to be transcribed. Developmental specificity could reflect either alterations to the complement of transcription factors between fetal and adult erythroblasts or be the result of a conformational change in the chromatin containing these genes, allowing differential presentation of the genes to the transcription machinery. Such a change need not occur at the time at which transcription begins but could be determined at an earlier stage in the cell’s maturational history, possibly as far back as the hematopoietic stem cell.

Support for the suggestion that γ and β genes may be in competition for expression comes from other HPFH mutations, which are due to single-base substitutions in the promoter of one or other of the γ genes. These mutants again result in high levels of expression of the affected γ gene (up to 50%) but not in addition to normal β output; rather there is a reciprocal relationship between the two. This effect cannot be due to competition for limiting trans-acting factors because the reciprocity only applies to the γ and β gene in cis while the chromosome in trans produces normal β output. Therefore, if there is competition it is likely to be for interaction with another cis-active sequence, for which the most likely candidate is the LCR.

Experimental evidence has recently been produced that is in agreement with the notion that globin genes may be in competition for expression. In the chick, a transient expression system was used to demonstrate competition between embryonic and adult β-like genes for access to the enhancer that lies between them. Transgenic mice have been used to show that developmental regulation of the human genes requires both genes to be present. In combination with the LCR, γ or β genes alone are expressed throughout development whereas when the region containing the γγ, δ, and β genes is intact, they are developmentally restricted in their expression; γ to the embryonic period, β to the fetal-adult stage.

We have investigated the relationship between human γ and β gene expression in adult mouse erythroleukemia cells

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(MEL) by cotransfecting a hamster adenine phosphoribosyl transferase (APRT) gene with constructs containing the human genes linked to one of the major hypersensitive sites on its own. MEL cells are blocked at a stage in erythroid maturation before globin gene transcription but can be chemically induced to undergo full erythroid differentiation and express human globin genes at a high level. We have demonstrated that these adult erythroid cells will readily transcribe both the human fetal γ-globin gene and the adult β-globin gene. No differential expression of γ and β genes was seen when they were attached to HS2, irrespective of gene order (HS2γγ or HS2βγ), and when HS2 is attached to the γγβ11δ and β genes in their normal chromosomal organization (but carrying the Greek HPFH −117 promoter mutation), transcripts from both γ genes and the β gene were readily detectable.

MATERIALS AND METHODS

Construction of recombinants. Recombinant fragments were constructed in the vectors pNXKN and pNB1.1 (a gift from R.W. Jones, University of Oxford), which contain a polylinker, including two Not1 restriction sites between the EcoRI and HindIII sites of pSP65 (Promega Corp, Madison, WI); pNB1.1 also has the 4.9-kb BglII fragment containing the human β gene ligated into the BamHI site in the polylinker.

A 1.9-kb PvuII/Kpn1 fragment containing HS2 from pGSE 272 was inserted into the Apa1 site of the β gene in pNB1.1, in both the genomic and reverse orientations to give pN2β15 and pN2β21, respectively.

A 3.2-kb HindIII/Xmn1 fragment containing the γ gene from pNHYH (a gift from R.W. Jones) was cloned into the XhoI site and the BglII sites of pN2β1.1 to give pN2βγ10 and pN2βγ11, respectively.

The recombinant pN2γ17 was constructed in pNXKN by the insertion of the HS2 restriction fragment into the XhoI site and the γ fragment into the KpnI site. All of these fragments (Fig 1) containing the genes linked to HS2 were released from the vector by digestion with Not1 and gel purified before electroporation into MEL cells.

The recombinant cos ME2.5 was constructed using a cosmid containing the 40-kb KpnI fragment encompassing the δ and β genes but with the −117 Greek HPFH mutation in the γ gene. The 1.9-kb HS2 fragment (see above) was ligated in the reverse orientation into the unique Smal site in the cluster, 3 kb 5' of the γ gene. The insert was released from the vector by an Asp718 digest, and the DNA was phenol extracted and ethanol precipitated before electroporation.

Isolation of stable transformants in MEL cells. The cells used were an APRT-negative, semi-adherent derivative of MEL line 585, cloned, and selected for a high level of inducibility before these experiments and maintained in RPMI 1640 + 15% fetal calf serum. Cells, 1 to 2 × 10⁶, were cotransfected with 1.0 μg hamster APRT (7.5-κb HindIII fragment, from pHaprt-1) and a 10-fold molar excess of the purified globin fragments, by electroporation at 400 V, 1,000 microfarads with laboratory-made equipment (R.W. Jones and G. Warner, University of Oxford). Selection for APRT positive cells was performed in medium containing methotrexate (10⁻⁵ mol/L), adenine (10⁻⁴ mol/L), thymidine (3 × 10⁻³ mol/L), and hypoxanthine (10⁻⁴ mol/L) after 24 hours. Cell colonies were counted after 5 to 7 days to give an estimate of the number of clones per pool.

Each flask was maintained in selective medium until a cell count of approximately 2 × 10⁶ was attained, when half the cells were harvested for DNA isolation and the remainder were induced with 5 mmol/L hexamethylene bisacetamide (HMBA) for 3 days before harvesting for RNA analysis. Individual clones of transfected cells were obtained by plating out low numbers of cells from pools in selective medium containing 1% methylcellulose in 24-well tissue culture plates. After 1 week of incubation, single clones were picked from each well with finely drawn Pasteur pipettes and grown up in selective medium. An MEL cell hybrid containing a human X-11 translocation chromosome (HU-11) was obtained from Dr A. Skoultchi (Albert Einstein College of Medicine, New York, NY) and was maintained in Dulbecco’s MEM containing methotrexate, hypoxanthine, and thymidine at the concentrations shown previously.

DNA analysis and estimation of gene copy number. DNA was isolated from the pooled cells and Southern blot analysis performed as described previously. For quantitation, a set of DNA controls was prepared by dilution of the construct pN2βγ10 in 5-μg

![Fig 1. (Top) The organization of the human β-globin complex, showing the DNase I hypersensitive sites (HS), according to the nomenclature of Tuan et al and the cosmid construct of Grosfeld et al containing 5' HS1 to 4, the β-globin gene and 3' HS1. (Middle) Fragments used in the present study to make constructs containing HS1 and various β-like globin genes. (Bottom) Constructs used in the present study. Arrows below the fragments denote their relative orientations.](https://www.bloodjournal.org/content/full/full/full)
aliquots of DNA isolated from MEL cells to represent two copies and eight copies per cell. These were standardized against human DNA. Five micrograms of DNA from each pool was digested with EcoRI (for γ gene quantitation) or with BamHI/EcoRI fragment from pNpl.1 or a 0.5-kb fragment from pNHyH). The blots were also hybridized with a 0.5-kb Pst I mouse α fragment from pUC18 as a control for the level of DNA per lane. Autoradiographs, representing 18-hour to 1-week exposures, were subsequently scanned using an LKB Ultrascan (LKB, Uppsala, Sweden) to determine the relative intensities of the human and mouse signals.

To analyze the organization of transfected DNA, a variety of digests were performed. The 4.9-kb BgII β-globin gene fragment, the 0.3-kb Bal I Xba I fragment containing HS2, and a 2.0-kb Pvu II APrTf 15 gene fragment were used in addition to the probes described above.

RNA analysis and quantitation. Total RNA was prepared 14 from cell pellets containing 2 to 5 x 10^6 cells. Human and mouse RNAs were detected using the quantitative RNase mapping procedure. 16 Three plasmids containing the SP6 promoter and the 5' ends of the genes were used as probe templates: (1) pSPpMxS (a gift from R.W. Jones), a 103-bp Pst I Bal I fragment corresponding to the 5' end of mouse α, linearized with HindIII, and giving a protected fragment of 93 nucleotides; (2) pGp1, a 174-bp BamHI fragment from pBpR containing the 5' end of the human β gene inserted into the Smal I site of pGEM I (Promega Corp), linearized with EcoRI, and giving a protected fragment of 135 bp; (3) pGp2, a 212-bp BamH1/MspI fragment from pBR322 containing the 5' end of the human γ gene ligated into the Smal I site of pGEM I, linearized with EcoRI, and giving a protected fragment of 142 bp. This probe does not distinguish γ and γ gene transcripts. Therefore, for this purpose pBCγ, a 516-bp HindII/Asa I fragment containing the 5' end of the γ gene ligated into the HindII/EcoRV sites of pBCSK+ (Stratagene, La Jolla, CA), was used (kindly provided by Dr M. Harvey, University of Sydney, Australia). The construct was linearized with HindII and transcribed from the T3 promoter, giving a protected fragment of 215 bp for γ and 131 bp for γ.

RNA probes labeled with α-32P-GTP were transcribed with SP6 polymerase (Amer sham International, Amer sham, UK) or T3 polymerase (Boehringer Mannheim, Mannheim, Germany) and approximately 1 x 10^6 cpm hybridized overnight with 2 μg total cellular RNA. Preliminary experiments demonstrated that these hybridizations were performed in considerable probe excess. After digestion with RNases A (40 μg/mL) and T1 (2 μg/mL), the protected fragments were separated on 8% acrylamide/8 mol/L urea gels and autoradiographed. Individual bands were quantitated by liquid scintillation counting after excising them from the gel, correcting for the number of G residues per protected fragment.

Analysis of globin chains. Globin chain synthesis was measured after incubating 2 x 10^6 cells with 50 μCi [3H]leucine in leucine-free incubation medium 20 made up with dialyzed fetal calf serum. Twenty milligrams of mouse and human hemoglobin was added to the lysed cells as carrier and globin was precipitated with acidified acetone and the chains separated by carboxymethyl cellulose chromatography 26 using a combined salt and pH gradient of 0.007 mol/L Na2HPO4, pH 6.4 to 0.028 mol/L Na2HPO4, pH 6.8. The incorporation of [3H]leucine was measured by liquid scintillation counting.

RESULTS
An intact β-globin cluster and an LCR β-globin construct produce high levels of β-globin mRNA in MEL cells. It has previously been demonstrated that hybrid MEL cells with a human chromosome 11 derived from human adult erythroid or nonerythroid cells produce only human β mRNA and protein and not human γ mRNA.21 We have confirmed this observation. In a cell line containing an X-11 translocation chromosome (HU-11, obtained courtesy of Dr A. Skoultchi), the level of human β mRNA averaged 44% of mouse α mRNA levels (Fig 2); because there is only a single β gene in those cells, this is equivalent to 88% of endogenous mouse levels. Hybridization of a 20-fold excess of RNA to a human γ probe gave no detectable band in an RNase protection assay, which, given the sensitivity of this technique, indicates that the level of γ gene expression is at least 500-fold less than β gene expression (data not shown). This confirms that MEL cells are capable of exhibiting stage-specific transcription of the human globin genes.

To provide a baseline against which to compare constructs containing the human β and γ genes, we first introduced the cosmid containing the intact β LCR attached to a β-globin gene (Fig 1) into MEL cells. On induction of hemoglobin synthesis, high-level human β gene expression was observed in 25 of 25 pools of cells (Table 1, Fig 2). The amount of β mRNA produced was quantitated relative to the endogenous mouse α mRNA and ranged from 2% to 143%, the variability depending on the copy number (see below).

Expression of human γ and β genes in MEL cells. We also measured the levels of human β-globin mRNA produced in MEL cells stably transformed with fragments containing only one of the LCR hypersensitive sites, HS2, attached to a human β-globin gene (Fig 1). High-level expression was again observed in all DNA positive pools, irrespective of whether HS2 was in the normal or reverse orientation with respect to the β gene (Table 1, Fig 2). Quantitation of the βα/αM mRNA ratios ranged from 11% to 105% but showed even greater variation when corrected for gene copy number, revealing an inverse relationship between copy number and expression per copy (see below).

A similar result was obtained with MEL cells transfected with a fragment containing HS2 attached to the human γ gene in the normal orientation (Fig 1). High-level expression was observed in all DNA positive pools with the γα/αM mRNA ratios being comparable to, or higher than, the βα/αM ratios seen with HS2 β constructs (Table 1, Fig 2) and also showing an inverse relationship between copy number and expression per copy. This result demonstrates that under these conditions, MEL cells can transcribe high levels of human γ mRNA, even though the gene in its normal chromosomal environment, is inactive 21 or silenced 22 when transferred to MEL cells.

MEL cells transfected with constructs containing HS2 linked to both the human γ and β genes arranged as either HS2 γ β or HS2 β γ (Fig 1) produced abundant amounts of both human γ and human β mRNA (Table 1, Fig 2). The average ratio of γ/αM mRNA was 33% and that of βα/αM mRNA was 31% in the HS2 γ β constructs. For HS2
Fig 2. Expression of human β- or γ-globin RNA in induced MEL cells. (A) Representative RNase protection assay using 2 μg RNA from transfected MEL cell pools hybridized with mouse α probe and either human β or γ gene probes. The constructs used to transfet the cells are shown above the respective lanes. Full-length probes are shown at the left and controls consisted of induced MEL cell RNA and human adult or cord blood reticulocyte RNA. (B) Expression of human globin RNA after transfection of MEL cells with constructs containing HS2 attached to both human γ and β genes. Also shown (last lane) is RNA from induced MEL cells containing a human X-11 translocation chromosome (HU-11).

βγ the relative values were 26% and 32%, respectively. Of 18 pools examined, the ratios of γ/β mRNA produced were approximately equal (within the limits of experimental error) in all cases except one. To demonstrate that this was not the result of averaging the values from several different clones in each pool, individual cells from several pools were cloned in methylcellulose and expanded and induced for analysis. Each clone also produced approximately equal amounts of human γ and β mRNA, irrespective of the gene order. The exception to this pattern was a single pool containing HS2 βγ in which the transfected DNA remained unarranged but in which only trace amounts of γ were detected.

Table 1. Expression of Various β-Globin Gene Cluster Constructs in MEL Cells

<table>
<thead>
<tr>
<th>Construct</th>
<th>No. of Pools</th>
<th>No. of Clones per Pool Mean (range)</th>
<th>βαM mRNA(%)</th>
<th>γαM mRNA(%)</th>
<th>Copy No.</th>
<th>% Human RNA per Copy Range</th>
<th>Estimated %* Human RNA per Single Copy</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS432β1</td>
<td>25</td>
<td>3</td>
<td>2-143</td>
<td>36</td>
<td>0.3-19</td>
<td>1-136</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>(1-9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS2β</td>
<td>9</td>
<td>17</td>
<td>16-77</td>
<td>50</td>
<td>0.2-8</td>
<td>2-195</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>(11-25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS2βγ</td>
<td>12</td>
<td>2</td>
<td>11-105</td>
<td>60</td>
<td>0.6-34</td>
<td>1-56</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>(1-3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS2 γ</td>
<td>13</td>
<td>6</td>
<td>53-220</td>
<td>102</td>
<td>6-72</td>
<td>2-26</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>(1-10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>HS2 βγ</td>
<td>11</td>
<td>49</td>
<td>1-106</td>
<td>31</td>
<td>&lt;0.5-36</td>
<td>3-58</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>(1-160)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS2 βγ</td>
<td>7</td>
<td>3</td>
<td>3-90</td>
<td>32</td>
<td>0.5-16</td>
<td>3-30</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>(2-6)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

*Estimated from graph of expression per copy versus copy number, as in Fig 5.
†HS2 in inverse orientation.
mRNA were detected while the β mRNA level was similar to that in the other pools.

The combined levels of human β and γ mRNA in these stable transformants averaged 65% of the endogenous mouse α mRNA levels, similar to the values obtained with either γ or β gene alone with HS2. Furthermore, when globin chain synthesis was measured in these cells, approximately equal amounts of human γ and β chains were demonstrated, in amounts approximately equal to the endogenous mouse β-chain synthesis and averaging about 50% of the mouse α-chain production (Fig 3).

We also cotransfected MEL cells with APRT and a construct containing HS2 inserted into the 5' end of the 40-kb Kpn I fragment containing the γ, γ, δ, and β genes in their normal genomic organization (Fig 1). The γ gene in this construct contained the G to A substitution at position -117 in the promoter, a change that produces an HPFH phenotype in vivo.16 Analysis of the RNA from induced cells resulted in readily detectable expression of the human globin genes in 20 of 20 pools (Fig 4A). The overall levels of expression (Table 2) relative to αγ were lower than those seen with HS2 γ, β, and HS2 βγ constructs, though the average copy number of the exogenous DNA per cell was high (18 to 63 copies). In each of the 20 pools, all three human genes were expressed. The relative proportions of γ, γ, and β mRNA were approximately 1:2:1 (Table 2).

Relationship of gene copy number to exogenous RNA production. In previous studies of the β-globin cosmid or HS2 constructs transfected into MEL cells, the level of RNA production was reported to be directly related to the copy number of the transfected genes.19,21,22 However, in our studies the level of human mRNA detectable in pools containing an average of one or more copies per cell varied over only a narrow range despite wide differences in copy number. When mRNA output per gene copy is plotted against copy number, an inverse correlation is obtained (Fig 5), suggesting that at high copy numbers, there is a reduction in the number of transcripts from each copy or that posttranscriptional processes limit the accumulation of globin mRNA. This analysis was performed on pools of transfected cells which, as a result of the cotransfection procedure, probably comprise mixtures of cells with and without the exogenous globin genes. Therefore, no conclusions can be drawn about the exact nature of this inverse relationship, which would require an analysis of a large number of individual clones containing variable numbers of the integrated genes. Nevertheless, the fact that such a relationship can be observed in pools suggests that extreme caution should be exercised in drawing conclusions about the level of expression per gene copy.

Inducibility and stability of transfected genes. In keeping with previous results,19,21,22 the constructs used in this study showed inducible expression in parallel with the endogenous mouse globin genes on treatment of the transfected cells with HMBA (data not shown). In several cases the transfected human genes showed high levels of expression in the uninduced cells, with only a small increase (1.5- to 4-fold) after induction. In these cells we presume that position effects allow high levels of transcription even though the endogenous genes are transcriptionally silent.

The stability of the transfected cells was examined by maintaining pools and individual clones of cells transfected with HS2γβ and HS2βγ in continuous culture for 9 months. RNA analysis at frequent time points throughout this period showed no differences in the proportions of human γ and β mRNAs (data not shown).

Organization of the integrated exogenous genes. Analysis of the DNA from transfected cells by Southern blotting demonstrated that in almost all the pools (51 of 52) and clones (10 of 10) examined, tandem head-to-tail repeats could be demonstrated. This was the case even in pools with a low overall copy number. Bands corresponding to head-to-head and tail-to-tail repeats were also seen in many samples, although at much lower levels.

Analysis of the cotransfected APRT gene showed a low copy number (~1) in all cases, with no tandem repeats. In the majority of cases the gene appeared to be integrated at a separate site from the globin genes. Thus, by using cotransfection, the potential problem of selecting sites of integration on the expression of a linked selectable marker has been obviated.

**DISCUSSION**

The results reported here confirm the importance of elements of the LCR for high-level expression of the β-like genes and confirm that one of these elements, HS2 at -10.7 kb, (previously known as HSII; or HS 3') can, to a large degree, substitute for the whole of the LCR complex in allowing high-level expression.19,21,22 Previous work15-28 has demonstrated that γ genes without an LCR can be expressed after transfection into MEL cells but only at a very low level and are presumably dependent on the site of integration; contradictory results were obtained as regards appropriate regulation of the gene on induction. We have
now demonstrated that there is no differential effect of HS2 on γ and β gene expression in MEL cells; HS2 attached to the γ gene alone produces mRNA expression levels as high, if not higher than when it is attached to the β gene alone. Furthermore, when constructs containing HS2 upstream of both genes are expressed in stable MEL cell transformants, both genes are expressed in high, approximately equal amounts, irrespective of the order of the globin genes. This high-level expression can occur, in some cases, before induction of the endogenous genes, which suggests that all the trans-acting factors necessary for globin gene transcription are present before induction and that there must be additional mechanisms responsible for the fact that before induction the endogenous genes are repressed while the activity of the exogenous genes depends on their site of integration.

With the construct containing HS2 inserted at the 5' end of the 40-kb fragment containing the αγγ-117 δ and β genes in their normal genomic organization, expression of the αγ, γγ, and β genes was readily detectable in all pools in an approximate ratio of 1:2:1. The increased γ gene expression may be due, in part, to the fact that the γ gene in this construct contains a known nondeletion HPFH mutation at position -117. In vivo, the relative expression of the three genes is 0.1:1:2.3; and, transfer of a chromosome 11 containing this mutated γ gene to MEL cells resulted in an expression pattern in which γ chain synthesis greatly exceeded β and γγ-chain production. While it will be necessary to confirm the expression results from the HS2γγγ-117β construct using a normal cosmid, we would stress that in MEL cells, the γγγγ and β genes are expressed at levels much more equal to each other than they are in vivo or after chromosome transfer.

Previous studies in transgenic mice or transfected MEL cells have produced variable results with respect to the copy number dependent levels of human β-globin gene expression.1,12,13,24,25 It is not yet clear to what degree copy number dependence may be influenced by whether an intact LCR, a microlocus containing the four hypersensitive sites, or just HS2 on its own has been used in these experiments. In our series, an inverse correlation between expression per gene copy and copy number was clearly demonstrated, a relationship that we also found in MEL cells transfected with constructs containing the major upstream regulatory region of the α-globin locus linked to an α-globin gene.32 It may be that while there is copy number dependent expression at the low end of the range, it is lost or even reversed at high copy numbers. It remains to be seen whether this effect is responsible for the lower levels of expression seen with the HS2γγγ-117β construct where consistently high copy numbers were observed.

Our results in MEL cells show no evidence of competition between the γ and β genes for expression. These results are significantly different from those obtained when related constructs are introduced into transgenic mice. Transgenic mice containing the human γ or β genes, with no LCR elements attached, show low-level, tissue-specific expression with developmental regulation.15,30 Attachment

Table 2. RNA Analysis of 20 Pools of MEL Cells Transfected With HS2γγγ-117β

<table>
<thead>
<tr>
<th>No. of Clones per Pool</th>
<th>αγγγ mRNA %</th>
<th>γγγγ mRNA %</th>
<th>ββ β β mRNA %</th>
<th>γγγγ mRNA %</th>
<th>γγγγ γ γ γ γ mRNA %</th>
<th>Copy No.</th>
<th>% Human mRNA per Gene Copy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>9</td>
<td>3.5 ± 1.8</td>
<td>7.5 ± 4.9</td>
<td>2.9 ± 1.3</td>
<td>3.8 ± 1.9</td>
<td>46 ± 11</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Range</td>
<td>5–14</td>
<td>0.4–7.5</td>
<td>0.8–22.7</td>
<td>0.8–6.5</td>
<td>0.3–7.9</td>
<td>18–63</td>
<td>0.2–1.6</td>
</tr>
</tbody>
</table>
of various elements of the LCR to either gene confers high-level expression but developmental stage specificity is lost.\textsuperscript{10,11,25,40} However, developmental regulation is restored when the intact LCR or the four hypersensitive sites are transferred to mice along with a fragment containing the $0.8$-$8.8$ region of the human cluster.\textsuperscript{16,11} This has led to the suggestion that competition between $\gamma$ and $\beta$ genes is a necessary facet of developmental regulation.

The lack of competition between the $\gamma$ and $\beta$ genes seen in our experiments after transfection of MEL cells could be due to the use of HS2 alone in place of the intact LCR or an abbreviated construct containing all four HSs. It is clearly sufficient to allow high-level expression of both genes, and while it might have been imagined that a single site is more likely to be selective than multiple sites, it is possible that a complex interaction between the various hypersensitive sites and the gene promoters is necessary for proper regulation.

It is also possible that the relative spacing of the LCR and the $\gamma$ and $\beta$ globin genes is important for their regulation, although this is made less likely by the fact that chromosomes with only a single $\gamma$ gene\textsuperscript{11} or with up to five $\gamma$ genes show normal developmental switching.\textsuperscript{11} Certainly, there was little difference in the expression patterns of the HS2$^\gamma$-$\beta$ and HS2$^\gamma$-$\tilde{\gamma}$-$\beta$ constructs to suggest that the organization of the various components in the system may be critical to their expression.

Both of these explanations are made further unlikely by the fact that both the HS2$^\gamma$-$\beta$ and HS2$^\gamma$-$\tilde{\gamma}$-$\beta$ constructs show developmental regulation in transgenic mice (unpublished observation). A major difference between using transgenic mice and transfected cells for examining globin gene expression is that in the former case, the injected DNA passes through a normal developmental history before expression of the genes in mature erythroid cells, while in the latter system the introduced DNA, although integrated into the mouse genome, can only form chromatin with the protein factors available in the MEL cell. It could be that the important trans-acting factors that determine stage specificity are not those present at the time of globin gene transcription but those present at an earlier stage in the history of an erythroid cell (eg, BFU-E, pluripotent stem cell), which could determine which gene will later be available for expression. Therefore, MEL cells might be permissive for expression of any transfected DNA containing appropriate erythroid cell signals. If this were so, it would explain why both $\gamma$ and $\beta$ genes are expressed in MEL cells after transfection but show developmental regulation in transgenic mice.

This explanation would also be consistent with the observation that MEL cell-human chromosome 11 hybrids ultimately express only the adult $\beta$ gene,\textsuperscript{21,44,45} except when the chromosome 11 is derived from HPFH patients when the $\gamma$ genes are expressed in the hybrid cells.\textsuperscript{30} These hybrid cells receive an intact chromosome, with its associated proteins, which has been through a normal developmental history. Therefore, MEL cells may be capable of perpetuating regulatory information associated with the transferred chromosome but be incapable of reconstructing such information on naked DNA. If this interpretation is correct, the important events in the developmental regulation of the globin genes may occur not in the recognizable erythroid cells but in an earlier precursor.

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